Development of Monoclonal Antibodies to Pear Psylla (Hemiptera: Psyllidae) and Evaluation of Field Predation by Two Key Predators

THOMAS R. UNRUH,[1] TIFFANY YU, LAURA S. WILLETT, STEPHEN F. GARCZYNSKI, AND DAVID R. HORTON

USDA–ARS, 5230 Konnowac Pass Road, Wapato, WA 98951

Ann. Entomol. Soc. Am. 101(5): 887–898 (2008)

ABSTRACT The pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), and related psyllids are important pests of pear (*Pyrus* spp.) worldwide. Many of these pests are thought to be partially controlled by predatory insects. To improve our understanding of the predator species that attack pear psylla, we developed monoclonal antibodies (MAbs) against this pest for predator gut content studies. Mice were immunized with homogenates of nymphal, adult, and egg stages of pear psylla. A mouse immunized with nymph homogenate showed high activity against all three antigen types and was used for MAb development. From 952 hybridomas screened, 35 showed good activity to pear psylla and low activity against nontarget arthropods. Four MAbs were retained: two from immunoglobulin M (IgM)-secreting hybridomas, both with high activity against all stages of psylla except young eggs, and two immunoglobin G-secreting hybridomas, both with high activity against psylla eggs and gravid adult females. Using one of the IgM-MAbs, pear psylla remains were detected in the predatory bugs *Anthocoris tomentosus* Péricart (Heteroptera: Anthocoridae) and *Deraeocoris brevis* (Uhler) (Heteroptera: Miridae) in laboratory feeding trials. Digestion half lives typically exceeded 24 h and were dependent on meal size and predator life stage. Gut content analysis of 970 field-collected *D. brevis* and *Anthocoris* spp. showed that the proportion which fed on psylla averaged 59% and that percentage closely tracked the density of pear psylla nymphs during three seasons. The utility of these antibodies for the study of trophic interactions and habitat management in relation to biological control of pear psylla is discussed.

KEY WORDS insect predation, monoclonal antibodies, enzyme-linked immunosorbent assay, gut contents

The pear psylla, *Cacopsylla pyricola* (Förster) (Hemiptera: Psyllidae), is a native pest of pears (*Pyrus* spp.) in northern Europe and an introduced and widely distributed pest of pears in North America. A closely related species, *Cacopsylla pyri* (L.), usually replaces *C. pyricola* as the dominant pest in southern Europe and closely related pest Psyllidae are found throughout temperate Eurasia (Horton and Unruh 2007). Damage caused by pear psyllids arises as honeydew excreted by the nymphs falls on to the fruit, leading to fruit marking and reductions in value. The pear psylla has demonstrated rapid evolution of resistance to insecticides increasing the difficulty to maintain integrated pest management programs for pear in both Europe and North America (Riedl et al. 1981).

Many predatory insects attack pear psylla both in North America and in Eurasia. These include several predatory bugs (Heteroptera) in the Anthocoridae and Miridae, ladybird beetles (Coccinellidae), and green lacewings (Chrysopidae) (Madsen et al. 1963; Nickel et al. 1965; McMullen and Jong 1967; Westigard et al. 1968; Herard 1986; Solomon et al. 1989; Unruh et al. 1994; Horton and Lewis 2000; Horton et al. 2002, 2003). In western North America and Western Europe pear production areas, these predators are often found in association with other potential predator species including various ants, the Mullein plant bug, *Campylomma verbasci* (Meyer) (Heteroptera: Miridae); the European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae); and several spider species (Gut et al. 1982, Lenfant et al. 1994, Horton et al. 2002).

Unfortunately, the relative importance of the various predator species in suppressing pear psylla is not clearly understood. Most field studies of pear psylla consist of measurements of psylla and predator abundances, which provide only correlative evidence of which predators are important in biological control. There are a few studies that describe specific predators' abilities to consume psylla in the laboratory (Brunner and Burts 1975, Hansen 1975), and several studies using field cages or open release of predators to demonstrate the capacity of predators to reduce

[1] Corresponding author: e-mail, thomas.unruh@ars.usda.gov.

psylla abundance (Sauphanor et al. 1993, Rieux et al. 1994, Unruh and Higbee 1994, Faivre-D'Arcier et al. 2001, Sigsgaard et al. 2006). There are no studies that allow us to confidently rank the relative importance of psylla predators across seasons and localities. To this end, we have developed monoclonal antibodies (MAbs) for the detection of psylla remains in predator guts to better measure the relative importance of predators associated with pear psylla. We follow methodological precedents of many similar studies for other target prey (Greenstone and Morgan 1989; Hagler et al. 1992, 1993, 1994; Fournier et al. 2006; others reviewed in Greenstone 1996; Symondson 2002). The monoclonal antibodies developed here complement our ongoing studies of predation of pear psylla in pear orchards in central Washington, particularly those designed to understand the importance of ground covers and nonorchard habitats as they affect natural enemy biology and biological control of psylla.

Materials and Methods

Test Insects. Pear psylla were reared on open pollinated seedlings of pear, *Pyrus communis* (variety Bartlett, = variety Williams; Rosaceae) in a greenhouse at the Yakima Agricultural Research Laboratory (YARL). In addition to pear psylla, we used several nontarget and predator species to determine specificity of monoclonal antibodies and for detection and digestion studies. These insects included *Anthocoris tomentosus* Péricart (Heteroptera: Anthocoridae); *Deraeocoris brevis* (Uhler) (Heteroptera: Miridae); Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), twospotted stinkbug, *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae), green apple aphid, *Aphis pomi* (DeGeer) (Hemiptera: Aphididae); and an unidentified Psyllidae found on antelope bitterbrush (*Purshia tridentata* (Pursh); Rosaceae). Colonies of *A. tomentosus* and *D. brevis* were reared on bell bean (*Vicia faba* L.; Fabaceae) seedlings infested with black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae); codling moths were reared on artificial diet (Hansen and Anderson 2006). Several other species were opportunistically tested for cross-reactivity and included two other Anthocoridae, *Anthocoris antevolens* White, and *Orius tristicolor* (White); one Coccinellidae, the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville; two Chrysopidae, *Chrysoperla plorabunda* (Fitch), and *Chrysoperla rufilabris* (Burmeister); the black bean aphid; and pear psylla honeydew. The lady beetles and *C. plorabunda* were purchased from Rincon-Vitova Insectaries (Ventura, CA); *C. rufilabris* and *Ephestia* were purchased from Beneficial Insectaries (Redding, CA). All other species were collected from nearby field sites, notably at the USDA–ARS Research Farm, 18 km east of Moxee, WA (Moxee Farm).

Antigen Preparation. For antigen production, clean pear seedlings were placed in a screened cage with adult psylla of both sexes. Psylla were allowed to oviposit for 3 d. Egg-infested leaves were taken from the plants and eggs were removed from the leaves. Each egg-infested leaf was submerged in tap water in a glass petri dish and a #1 artist paint brush, with its bristles trimmed to 5 mm, was used to gently free the psylla eggs from the leaf into the water. Eggs were then concentrated onto a Whatman #3 filter paper disks using a Büchner funnel. After rinsing and removal of debris, the eggs were allowed to air dry and then transferred into 2-ml cryo-vials and stored at −80°C. Third through fifth instar psylla nymphs were individually picked from infested pear seedlings using a dull probe, rinsed in water, and transferred to dry on a Whatman #3 filter in a petri dish. Nymphs were frozen and transferred to cryo-vials and stored at −80°C. Adult psylla were collected from pear orchards using a beat tray and aspirator. Males were separated from females in the laboratory, transferred into cryo-vials, and stored at −80°C. Female psylla were not used in antigen preparation, because mature females were likely to contain developing or fully developed eggs.

Stage-specific antigens were prepared by homogenizing psylla by stage in 1.5-ml microfuge tubes. Approximately 5 mg of a single psylla stage was placed in a tube and powdered with a plastic pestle over liquid N_2. Next, 1 ml of 1× phosphate-buffered saline (PBS), pH 7.2, was added and the contents homogenized with a sterile plastic pestle. The tube was then spun at 14,000 × *g* for 10 min, and the clear supernatant was transferred to a clean tube. Six such tubes for each life stage were combined in a sterile 10-ml Falcon tube, mixed by inversion, and reallocated to clean 1.5-ml tubes to ensure equal concentrations. Protein concentrations of the homogenates were estimated using the Bradford assay (Bradford 1976) modified for a microplate (reagent 500-0205, Bio-Rad, Hercules, CA). Protein concentrations of the stocks used for immunizations were 2.35, 1.69, and 1.95 μg/μl for psylla adult males, nymphs, and eggs, respectively.

Antibody Development. Antibody production and initial hybridoma screenings were done at the Monoclonal Antibody Center, Department of Veterinary Microbiology and Pathology (DVMP) at Washington State University (WSU) (Pullman, WA). MAbs were further tested at the USDA–ARS YARL. Below we briefly outline the methods used for immunization, hybridoma production and isolation, and enzyme-linked immunosorbent assay (ELISA).

Twelve BALB/c mice, in four groups of three mice, were injected with different antigens corresponding to different psylla life stages as follows: 0–48-h-old eggs, fourth–fifth instar nymphs, adult males, and a combination of these three antigen sources. Four subcutaneous injections consisted of 50 μg of antigen in 200 μl of adjuvant. Mice were immunized at 44, 65, 86, and 109 d after birth. Serum samples derived from tail bleeds taken before each immunization were tested for anti-psylla antibody titers by using indirect ELISA

with the four antigens used for injection (see below for detailed ELISA protocols). A single mouse was selected for fusion based on high response to the three antigen types. Its spleen cells were fused with X63 Ag0.688 mouse myeloma cells by using standard methods (Hagler et al. 1992, 1993, 1994). Fused cells were dispersed into ten 96-well plates at near limiting dilution. After adequate growth, their supernatants (1:50 dilutions) were used as the primary antibody in indirect ELISA with adult, nymph, and egg antigens. The number of isolates was reduced to 34 based on the initial ELISA results, and four isolates were chosen based on additional screening against psylla antigens and against nontarget antigen sources, including pear leaves, pear psylla honeydew, other herbivorous insects and several predator species. The four chosen hybridoma lines (21, 28, 29, and 34) were isotyped (#iso2, Sigma-Aldrich, St. Louis, MO) and one anti-psylla egg antibody (line 29) and one anti-psylla nymph (line 34) were expanded through ascites production under contract with the WSU-DVMP. Three replicates of each of these four hybridoma lines are cryogenically preserved at WSU-DVMP and at YARL.

Indirect ELISA. Indirect ELISA methods were multiply optimized, first for screening of supernatants from hybridomas lines and then more thoroughly for the ascites. For tests of nontarget herbivores and predators, insects were homogenized in 100–1,500 μl of PBS, depending on their size (≈1:20 wt:vol, insect/PBS); 50 μl of insect homogenate (hereafter antigen) was loaded individually into wells of 96-well polystyrene microtiter plates, covered tightly with Parafilm, placed into ZipLoc bags, sealed, and incubated at 4°C overnight or at 37°C for 2 h. After incubation, the antigen was removed and each well was filled with 300 μl of milk blocker (2% nonfat powdered milk in distilled H_2O), covered, and then incubated 1 h at 37°C. Blocker was removed and plates were washed using a standard format (three washes with PBS plus 0.05% Tween 100 and two washes with PBS alone). After washing, 50 μl of antibody (1/1000 ascites fluid in 1%milk, 0.05% Tween 100 in H_2O or 100 μl of serum or culture supernatant 1:50 in PBS) was added to each well, covered tightly, and incubated for 1 h at 37°C. Antibody was removed and washed followed by the addition of 50 μl of 1:1000 goat anti-mouse alkaline phosphatase enzyme conjugate (μ-chain. A-9688, Sigma-Aldrich; 1% milk, 0.05% Tween 100 as diluent; with supernatant primary we used no Tween in diluent) and incubated 1 h at 37°C. Conjugate was removed followed by washing and 100 μl of substrate solution (1.0 mg/ml *p*-nitrophenyl phosphate substrate [Sigma N1891] in 1 M diethanolamine, pH 9.8) was added to each well. Absorbance of enzyme product was read at 30, 60, and 90 min at 405-nm wavelength with a microplate auto-reader (model EL311, BioTek Instruments, Winooski, VT).

The overall ELISA protocol for the preliminary screening of putative MAbs by using supernatants and evaluating mouse serum was as described above, except that Tween 100 was used only in the washes. In preliminary screening of serum from tail-bleeds and supernatants of putative hybridoma clones, positive test antigens were the same as those used for immunization in 1:200 dilutions. The primary antibody consisted of 1:50 dilutions of serum or "grow-to-die" (GTD) supernatant from hybridomas. Nontarget evaluations used GTD supernatant as antibody and clarified homogenates of the arthropods listed above as negative controls, ensuring that they had no previous contact with pear psylla. Positive controls consisted of 1:200 dilutions in PBS of a psylla adult; negative controls consisted of the same species of predator as being tested but with no history of psylla feeding. Ascites of MAb34 was used for evaluating all feeding trials and field collected predators.

Western Blot Analysis. Soluble proteins were extracted from eggs, nymphs, adult males, or adult females by homogenization in PBS containing a protease inhibitor cocktail (Complete Mini, Roche Applied Science, Indianapolis, IN). The homogenates were clarified by centrifugation for 10 min at 20,000 × *g* in a microfuge. Protein concentrations were determined using the Bio-Rad Protein assay by using the microassay with bovine serum albumin as the standard. Polyacrylamide electrophoresis and transfer to polyvinylidene difluoride (PVDF) filters were done using the NuPAGE Bis-Tris electrophoresis system (Invitrogen, Carlsbad, CA) as follows. Soluble proteins (10 μg) were denatured with NuPAGE LDS sample buffer and reducing agent (Invitrogen; 106 mM Tris-HCl, 141 mM Tris base, 2% LDS, 10% glycerol, 0.51 mM EDTA, 50 mM dithiothreitol, 0.22 mM SERVA blue G250, and 0.175 mM phenol red, pH 8.5) and heated at 70°C for 10 min. After treatment, the denatured protein samples and molecular weight (MW) markers (Magic-Markers XP, Invitrogen) were separated on 4–12% NuPAGE Novex Bis-Tris gels (Invitrogen) by polyacrylamide gel electrophoresis in 3-(*N*-morpholino)-propanesulfonic acid (MOPS) SDS running buffer (50 mM MOPS, 50 mM Tris base, 0.1% SDS, and 1 mM EDTA, pH 7.7) and then tank transferred to methanol-activated PVDF filters (OWL Separation Systems, Portsmouth, NH) in NuPAGE transfer buffer (25 mM Bicine, 25 mM Bis-Tris [free base], and 1 mM EDTA, pH 7.2) containing 20% methanol. After transfer, the PVDF filters were rinsed with Tris-buffered saline (50 mM Trizma base and 150 mM NaCl, pH 7.5) containing 0.1% Tween 20 (TBST) and then blocked with a solution of 3% dry milk (Bio-Rad) and 2% goat serum (Sigma-Aldrich) in TBST at 4°C, for 1 h. The monoclonal antisera (MAb34) or (MAb29) were added directly to the blocking solution (final dilution 1:1,000) and incubated at 4°C overnight. After rinsing with TBST, the blots were washed four times, 20 min each, with fresh TBST, and then transferred to fresh blocking solution. Secondary antibody (goat anti-mouse immunoglobulin [Ig]G or IgM conjugated to horseradish peroxidase, Sigma-Aldrich) was added to a final dilution of 1:10,000 (vol:vol) for incubation 2 h at room temperature. After washing four times, 20 min each, with fresh TBST, the immunoreactive proteins were detected with the ECL Western Blotting System (GE Healthcare, Little Chalfont, Buckinghamshire,

United Kingdom), and the chemiluminescence was visualized with the Alpha Innotech (San Leandro, CA) imaging system.

Feeding Trials and Assessments of Predation in the Field. To demonstrate the sensitivity of the MAb34 antibody for detection of recent feeding on psylla by predators we determined the time duration after a predation event over which psylla could be detected in the guts of two predatory bugs, *A. tomentosus* and *D. brevis.* Our purpose was to test how probability of detection was affected by four major factors: predator species, predator life stage (adult or nymph), meal size (one or three psylla nymphs), and time since last feeding. Before conducting a feeding test, bugs were individually isolated in four dram glass vials with a piece of clean bean leaf (to provide liquid nourishment and maintain humidity) and were kept without insect food for 48 h. Subsequently, feeding by individual predators was observed in a transparent arena consisting of a sandwich of three pieces of 0.5- by 6- by 10-cm Perspex with the middle piece of Perspex having a 4-cm-diameter hole in its center. The top layer of the arena was opened and two to five fifth instar psylla nymphs were added. After prey addition, a large nymph or adult of *A. tomentosus* or *D. brevis* was added. The predators in 10–15 arenas were monitored and when each predator finished its final prey item (either its first or third prey) the predator was moved from the arena to a clean 1.5-ml microfuge tube. Predators were then frozen immediately at −20°C or allowed to digest the prey (without additional feeding on a nontarget food) for a fixed period of time before freezing. The feeding assays were conducted at room temperature (21–23°C) under continuous light.

Field-collected predators also were assayed, to assess whether positive scores (i.e., evidence of having fed on pear psylla) tracked seasonal changes in densities of pear psylla nymphs. *Anthocoris* spp. and *D. brevis* were collected from an unsprayed pear orchard by using beating trays and aspirators. After collecting 10–20 predators, the aspirator vial was placed in an ice chest and kept cool until returned to the laboratory and then frozen at −20°C. On dates that predators were collected, 50 pear leaves were also collected from the orchard and returned to the laboratory to determine the number of psylla nymphs per leaf. More than 900 bug predators were collected on eight dates in 2004, seven dates in 2005, and five dates in 2006.

Before homogenization for ELISA, predators from digestion and field studies were removed from tubes in which they were frozen, rinsed briefly in clean tap water, blotted dry, and moved to a clean 1.5-ml tube for homogenization. The water rinse was used because pilot studies showed that prey signal can be detected in predator feces (100-μl rinses of three of four tubes where *D. brevis* had digested psylla for 24–32 h proved positive by ELISA) and in honeydew (10 of 88 *D. brevis* that had never been in contact with psylla when homogenized with 1 μl of fresh psylla honeydew were positive by ELISA). We saw no evidence of false positive arising from rinses with tap water. Predators were homogenized in 100 μl of PBS by using a sterile plastic pestle and 50 μl of the homogenate was used in the ELISA as described above. Homogenates from three or more starved predators and three or more PBS control wells were used as negative controls in each plate.

Data Analyses. Absorbance readings at 405 nm from ELISA by using mouse serum and hybridoma supernatants were corrected for absorbance in PBS control wells and are presented graphically. For feeding trials and field-collected predators, ELISA values were deemed positive when the optical density readings were four standard deviations above the mean for the control insects (starved conspecific predators) providing a conservative estimate of the positive threshold (Sutula et al. 1986). The digestion data were analyzed with generalized linear mixed model for a binomial response variable (i.e., yes/no scores from the ELISA) in PROC GLIMMIX (SAS Institute 2002–2004, Littell et al. 2006), with time of digestion in hours included as a continuous variable and predator stage (nymph or adult) and meal size (one or three nymphs) included as class variables. A stepwise method was used to fit the models, beginning with the three-way interaction (digestion time × meal size × predator stage) and all two-way interactions. Nonsignificant interactions involving the continuous variable (digestion time) were then discarded. The fitted models were then used to obtain least squares means for each combination of variables and the fitted curves were used to provide estimates of the half-lives. Data for the two predator species were analyzed separately.

Results

Immunity Development, Hybridoma Production, and Testing. The mice injected with pear psylla antigens developed immune responses to psylla antigen as measured by ELISA. The final tail bleed showed that the response levels generally corresponded to the antigen used to immunize the mice (Fig. 1). Relatively higher responses to egg or adult antigen were observed in mice immunized with egg or adult homogenates, respectively. However, serum antibody responses of mice immunized with nymph antigens showed less consistent patterns and the responses of mice immunized by the composite antigen was clearly greatest to the egg antigen. Importantly, a significant mouse to mouse variation in response intensities within all immunization classes was observed (Fig. 1). Based on relatively high responses to the three discrete antigen types, mouse nine was selected for monoclonal antibody production. In addition to showing the highest response to nymph and adult (male) psylla antigens, mouse nine serum showed the highest absorbance with egg antigens except for mice immunized with pure eggs.

In total, 952 hybridoma cultures were created in the fusion from mouse nine spleen cells. These were reduced to 35 cell lines based on their reactions with the egg, nymph or adult psylla antigens. Four cell lines were ultimately selected for cloning by dilution based on their high responses to psylla antigens and low

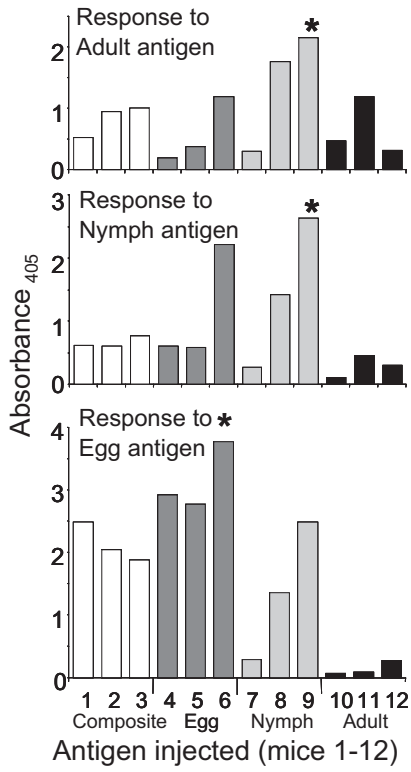

Fig. 1. Reactivity of serum antibodies from 12 mice to three psylla antigen sources in relation to antigens used to immunize mice as measured by absorbance values in indirect ELISA. A 1:50 dilution of serum was used as the primary antibody. The highest absorbance to a particular antigen is identified with an asterisk (*).

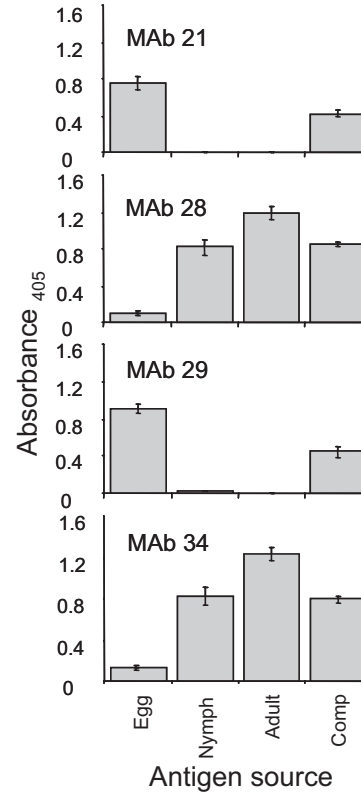

Fig. 2. Absorbance readings from ELISA for four putative monoclonal antibodies to pear psylla antigens by using 1:50 dilutions of supernatant as primary antibody in the indirect ELISA (mean ± 1 SE). Comp, composite.

responses to nontarget insects including predators. Figure 2 summarizes the response of antibodies derived from these four monoclonal cell lines against the same purified psylla antigens we used for immunizations. Two MAbs, 28 and 34, were IgM isotypes and showed high activity and affinity to all stages of pear psylla except young eggs. MAb21 and 29 were IgG isotypes and showed high and selective activity against pear psylla eggs and gravid adult females. These were not tested against eggs of other psyllids. Like many IgMs, MAb28 and 34 showed significant nonspecific binding in preliminary ELISA tests (not shown), which was controlled by addition of Tween 100 (0.05%) to several steps of the optimized ELISA protocol as described in the materials and methods.

Multiple nontarget insect species were tested using GTD supernatant in the optimized ELISA by using the four MAbs to demonstrate specificity to pear psylla and another psyllid species, and lack of response to nontarget species (Fig. 3). The MAbs showed affinity only to the two members of the family Psyllidae with only slightly higher activity to *C. pyricola* compared with the unidentified psyllid from bitter-brush. These results suggest that these MAbs may be useful for not only *C. pyricola*, but also congeneric species such as *C. pyri* and *C. bidens* in the Old World and other pest *Cacopsylla* spp. from other geographic areas. Because of similarity in the response profiles to the various antigens of MAb28 and 34 (IgM) compared with MAb21 and 29 (IgG) (Figs. 2 and 3), we suspect that these represent two duplicate pairs. Hence, ascites were made only to MAb29 as the anti-egg IgG and MAb34 as the anti-nymph IgM classes. Subsequent studies using ascites of MAb34 found no significant cross-reactivity with *A. antevolens*, *O. tristicolor*, *H. convergens*, *C. plorabunda*, *C. rufilabrus*, and *A. fabae* (data not shown), and we used MAb34 exclusively for digestion studies, and field evaluations (see below).

Detection of Psylla Antigens by Western Blot Analysis. Ascites fluid containing MAb29, an IgG-specific to psylla eggs, and MAb34, an IgM specific to all psylla stages, were used on immunoblots to visualize the protein(s) recognized by the antibodies. A specific protein of 37 kDa was detected consistently with MAb29 in extracts from eggs and gravid females (Fig. 4A). We suspect that the protein detected in gravid females is derived from the eggs contained within the abdomen. In contrast, MAb34 reacted with several protein bands in eggs, nymphs, and adult males and gravid females, making interpretation of these results speculative (Fig. 4B). Overall, the results of the im-

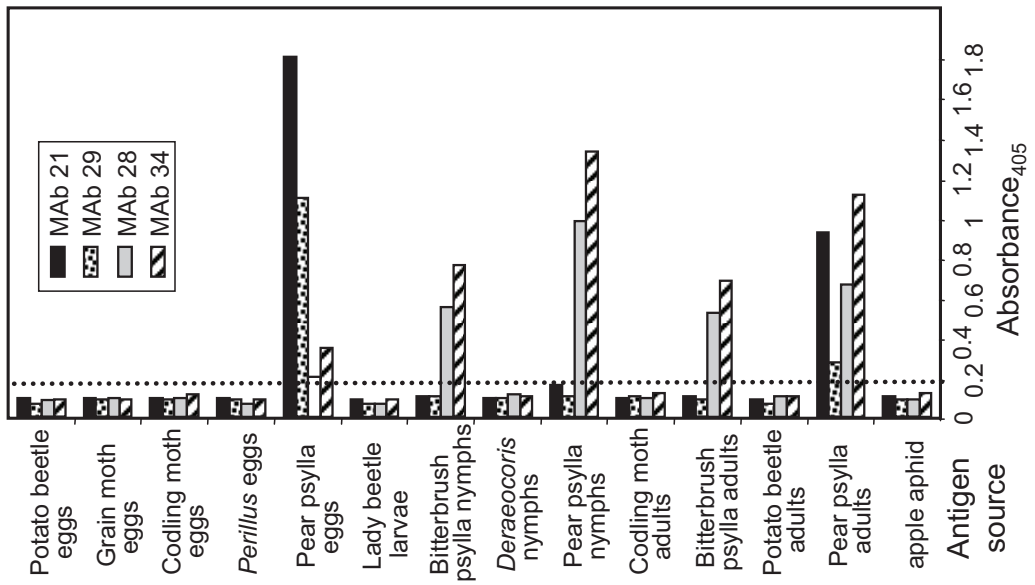

Fig. 3. Bars depict absorbance readings in indirect ELISA with four putative MAbs used as primary antibody and antigens from nine insect species. Grow to die supernatants were used as the primary antibody source; the dotted lines represents the mean plus 3 SD calculated over all nonpsyllid insects assayed.

munoblots are consistent with the specificities obtained by ELISA (Fig. 3), that is, MAb29 reacts specifically with psylla egg and MAb34 reacts with all stages, especially nymphal and adult stages.

Factors Affecting Prey Detection. Proportion of predator specimens scoring positive for psylla remains declined significantly with increasing digestion time in both *A. tomentosus* and *D. brevis* (Tables 1 and 2; Fig. 5) as detected by ELISA with MAb34. Both meal size and predator stage affected the probability of detecting the prey signal (Tables 1 and 2). Larger meals delayed loss of signal in both predators, as shown in Fig. 5 and by least squares means estimated from the models in Tables 1 and 2. Thus, mean ± SEM proportion of *D. brevis* scoring positive for the prey signal was 0.39 ± 0.06 for one prey consumed and 0.63 ± 0.08 if three prey were consumed. In *A. tomentosus*, the estimates are 0.56 ± 0.07 and 0.80 ± 0.05, for one and three prey, respectively. Predator life stage also significantly affected whether the prey signal was detected. For both species, probability of detecting the prey signal was higher in nymphal predators than adult predators (Tables 1 and 2; Fig. 5): 0.30 ± 0.07 versus 0.72 ± 0.08 for *D. brevis*, and 0.52 ± 0.07 versus 0.82 ± 0.05 for *A. tomentosus.* A significant interaction between meal size and digestion time for *D. brevis* (Table 1) was due to the rapid drop in signal beginning at ≈24-h digestion in predators that consumed three prey, relative to the much flatter curve shown in predators that consumed one prey (Fig. 5). The half-life of the prey signal varied between ≈28 and 50 h for *A. tomentosus* (depending upon predator stage and number of prey consumed) and between 4 and 38 h for *D. brevis* (Fig. 5).

Gut Content Analysis (GCA) of Field-Collected Predators. Gut contents were analyzed by ELISA in 438 *Anthocoris* spp. individuals and 532 *D. brevis* individuals collected over three growing seasons by using the MAb34 IgM antibody (Fig. 6). The proportion of the two predatory bugs that showed psylla signal averaged 59% for each species and closely tracked the abundance of pear psylla in the orchard through the three sample years (Fig. 6). Even when pear psylla nymphal densities were below one psylla nymph per leaf, predator gut contents often seemed highly positive (4–55%, depending on date). The large percentage values observed in spring may reflect predator feeding on psylla eggs, because this stage was very abundant at that time (data not shown); MAb34 readily detects eggs, albeit less strongly than it detects other stages.

Discussion

The effects of predators on insect populations are often difficult to estimate because predators leave little evidence. Approaches to demonstrate predation by specific species, to estimate the effects of predation on prey population trajectories, and to quantify insect predation broadly include (Luck et al. 1988) direct observation of predation events (e.g., Pfannenstiel and Yeargan 2002), declines in pest densities after releases of predators (reviewed in Sigsgaard et al. 2006), and morphological or biochemical estimation of gut contents (Harwood and Obrycki 2005). Of these approaches, only predator release studies have been used previously with pear psylla.

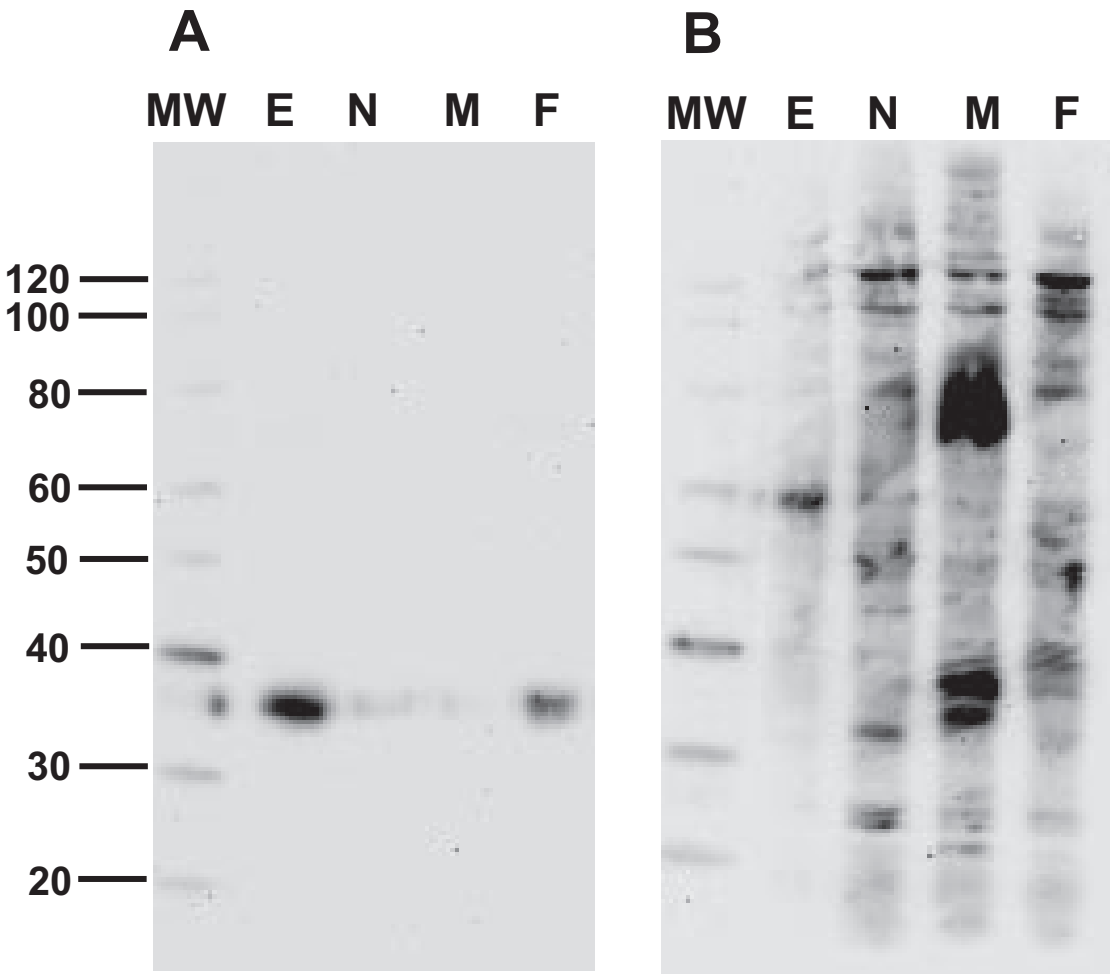

Fig. 4. Immunoblot detection of soluble proteins extracted from various psylla life stages. (A) MAb29 detection of soluble proteins extracted from eggs (E), late instar nymphs (N), adult males (M), and adult females (F). (B) MAb34 detection of soluble proteins extracted from eggs (E), late instar nymphs (N), adult males (M), and adult females (F). Size markers (MW) were MagicMarkers XP and apparent molecular mass in kilodaltons are to the left.

Because of pear psylla's small size and the diversity of predators which attack it, direct observation and manipulation approaches are arguably less efficient and more demanding in the field compared with GCA (Harwood and Obrycki 2005). Morphological identification of gut contents has been used for >100 yr for large predators with chewing mouthparts and remains the largest body of work for GCA of carabids and other large beetles. Recently, there has been a shift to biochemical methods for insect GCA (Sheppard and Harwood 2005, Harwood and Obrycki 2005); such meth-

Table 1. Effects of predator stage, number of prey consumed, and digestion time on detection of prey signal in *D. brevis*

Effect	Numerator df	Denominator df	*F* value	*Pr* > *F*
Stage	1	137	13	0.0004
Eaten	1	137	9.49	0.0025
Stage × eaten	1	137	2.32	0.1303
Digest	1	137	25.79	<0.0001
Digest × eaten	1	137	6.35	0.0129

We assayed 143 predators and included both adult and nymphs that fed on either one or three psylla nymphs. Digestion times included 0, 8, 16, 24, 32, 40, and 48 h. See Fig. 5 for fitted models and observed values.

Table 2. Effects of predator stage, number of prey consumed, and digestion time on detection of prey signal in *A. tomentosus*

Effect	Numerator df	Denominator df	*F* value	*Pr* > *F*
Stage	1	140	10.78	0.0013
Eaten	1	140	6.84	0.0099
Stage × eaten	1	140	0.34	0.5605
Digest	1	140	27.07	<0.0001

We assayed 145 predators and included both adult and nymphs that fed on either one or three psylla nymphs. Digestion times included 0, 8, 16, 24, 32, 40, and 48 h. See Fig. 5 for fitted models and observed values.

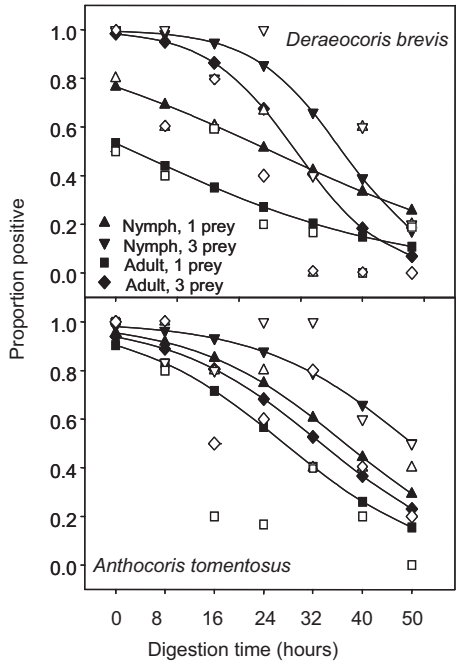

Fig. 5. Fitted model predictions (curves with filled symbols) and observed data (open symbols) for proportion of *D. brevis* (top) and *A. tomentosus* (bottom) scoring positive for the pear psylla signal, expressed as a function of digestion time. Factors considered in the model were digestion time, one or three pear psylla fifth instar nymph(s) consumed, and predator life stage (see text). Statistics for fitted models are shown in Tables 1 and 2. The dotted line represents the 50% detection level. Five or six predators were assayed for each unique combination of factors.

ods are necessary for GCA with predatory bugs and lacewings, because these predators ingest prey fluids, and may even liquefy a prey specimen before it is ingested (Cohen 1995). A variety of biochemical methods have been used in the field (Symondson 2002, Harwood and Obrycki 2005) with serological detection using prey-specific polyclonal or monoclonal antibodies represented most widely. More than 50 yr ago, Dempster (1960) used the precipitin antibody test to screen many thousands of field-collected predators for the presence of egg antigen of the broom beetle, *Phytodecta olivacea* Forster, to better understand the trophic dynamics of the arthropod community on Scotch Broom, *Cytisus scoparius* (L.). Subsequently, many workers have developed monoclonal antibodies for insect predator GCA, but only a few of these have analyzed significant numbers of predators to study ecological relationships in the field. Recently, Hagler and Naranjo (2005) estimated the influence of insecticide use on predator feeding frequencies on the whitefly, *Bemisia tabaci* (Gennadius), in cotton, *Gossypium hirsutum* L., fields (>30,000 specimens). In an earlier study (Hagler and Naranjo 1994, Naranjo and Hagler 1998), they estimated the relative importance of two coleopteran predators attacking whitetly and pink bollworm, *Pectinophora gossypiella* (Saunders), eggs in cotton. Recently, Harwood et al. (2007) used a dipteran-specific MAb to measure the influence of alternative host availability on the on spider feeding preferences for dipteran prey.

Also recently we have seen a significant number of studies using polymerase chain reaction (PCR) to detect prey DNA for predator GCA. Both serological and PCR-based approaches to GCA are extremely sensitive, and both have proven valuable in ecological investigations (Symondson 2002). With available or easily obtained DNA sequences from the prey and several nontarget taxa, prey-specific DNA primers can be designed and GCA studies by using PCR can begin. However, the costs of DNA extraction and PCR remain high, >$2.00/specimen (excluding labor) in our laboratory. In contrast, to develop monoclonal antibodies, as we have done here, requires many months and significant expenses in both materials and labor associated with care and use of the vertebrate sources of the antibodies, screening thousand of putative clones, and ELISA optimization studies. Another caution, the responses of the mice to the immunizations with crude psylla homogenates were idiosyncratic, as seen in Fig. 1, suggesting there is a nontrivial probability of failure when developing MAbs, particularly if only one or two mice were used. Thus, there is a much higher investment to develop MAb(s) than develop and test PCR primers, suggesting PCR may be better suited for smaller, or more descriptive studies, such as clarifying the roles of the species in a trophic web. Multiple PCR primers to amplify psylla-specific DNA sequences were developed by Unruh and co-workers and have been tested in various ways (Agustí et al. 2003b; T.R.U., unpublished data). However, we found that PCR was too expensive for larger field studies such as seasonal dynamics of feeding by specific predator species (i.e., Fig. 6), estimating relative impacts of key predators, or evaluating habitat perturbations that require analyzing many specimens. The field study presented here required only a few hundred dollars and <2 wk to homogenize and assay the specimens by ELISA. In sum, once developed and optimized, ELISA using MAbs can be far superior to PCR for high-throughput field studies, because it is more rapid than PCR, less critical in execution, and far less expensive per specimen.

Although ELISA provides a measure of target antigen concentration, this information has not proven useful in quantifying predation rates because the strength of the prey signal depends both on meal size and time of the last feeding event, as shown in Fig. 5 (see also Hagler and Naranjo 1996, Naranjo and Hagler 2001). The signal is also dependent on the ratio in sizes of predator and prey, i.e., with large predators, overloading of the wells in the ELISA plate with predator protein can create competition for binding sites between prey antigens and predator proteins (Hagler et al. 1997). Other factors that may influence antigen detection by ELISA include feeding efficiency and digestion rates, species differences, predator life stage, sex, and physiological condition, and prey life stage or size (Lovei et al. 1985, 1987, 1990; Hagler et al. 1992,

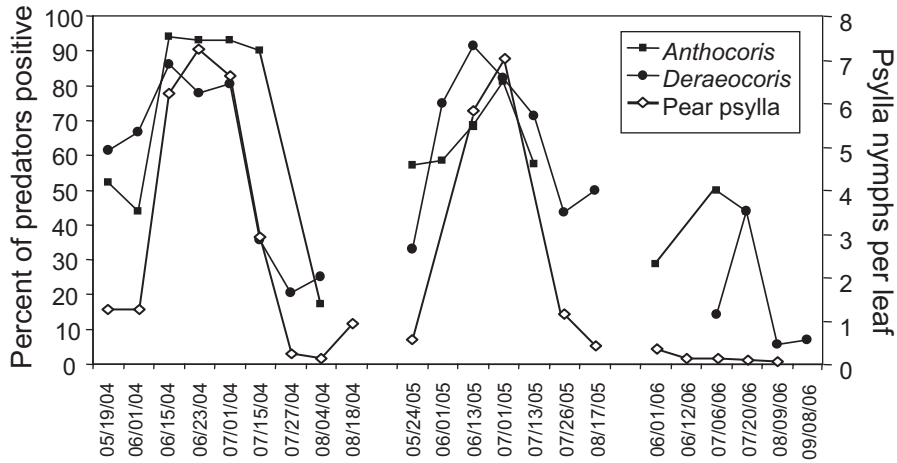

Fig. 6. Curves depicting observed percentages of predators (*D. brevis* and *Anthocoris* spp.) scoring positive for the pear psylla signal (left axis), across three seasons in relation to densities of pear psylla nymphs (right axis) are shown. Data for adult and nymphal predators were pooled within species. Ten or more predators were tested for each datum.

1993, 1994). Four of these factors (species, digestion time, meal size, and predator life stage) were shown to be important in our laboratory study with *A. tomentosus* and *D. brevis.* Digestion rate of prey signal is also well known to be a function of temperature (Hagler and Naranjo 1997), but this variable was not tested in this study. The temperature at which we measured digestion in the two bugs approximated the average daily temperatures observed for July and August in Yakima, WA; we would expect longer digestion half-lives in the cooler temperatures of spring and fall.

Overall, the relatively high percentage of positive detection in these two bugs may reflect both these long digestion intervals and the fact that they may consume many prey each day (Brunner and Burts 1975, Unruh and Higbee 1994). A second factor that might account for higher than expected positives in the predators is the potential for prior predation on another psyllid, such as that found on bitterbrush, before entering the study orchard. We were aware of this potential and are confident that nonorchard plants and trees with their attendant psyllids were not abundant enough near our study site to be of concern. Furthermore, at least half of the predatory bugs we assayed were nymphs and could not have moved from distant sources. A last factor that may have increased detection frequency is secondary predation, that is, bugs preying on other predators which in turn were full of psylla. Previous studies indicate this bias can occur under special conditions (Harwood et al. 2001, Sheppard et al. 2005), but those same studies suggest it would not occur at a high frequency. For now, we discount both nontarget prey and secondary predation as important biases in our studies.

The two bug species we assayed are relatively small, but pear psylla is also attacked by larger predators, including ladybird beetles, lacewings, and especially earwigs (Unruh et al. 1994). We have not yet developed double antibody sandwich ELISA for our pear psylla antibodies, but given the potential importance of larger predators in this system it may significantly increase the sensitivity of our assays (Hagler et al. 1997, Fournier et al. 2006). When considering all the limitations of ELISA for GCA, we infer that its least problematic use lies in measurement of the proportion of predators that have fed on prey in comparative studies as described below. However, various approaches to correct for the confounding of meal size and time since feeding through the use of functional response models (Naranjo and Hagler 2001) or by using estimates of antigen concentration (Sopp et al. 1992) may provide useful increases in resolution, particularly where an objective is to rank the relative importance of two or more predators.

Recently, GCA has supported fairly direct testing of ecological hypotheses. Harwood et al. (2004) used a MAb to test how the level of predation (as measured by the presence of aphid signal) in two taxa of spiders was influenced by the availability of alternative collembolan prey. Spiders showed higher rates of predation on aphids (more frequently positive) during periods of low Collembola density. Harwood et al. (2004) also saw differences between spider taxa: Eriogoninae were more responsive to collembolan densities than were Linyphiinae. However, they also proposed that although the Collembola may represent a sink in the short term, these prey are a resource that helps maintain spider densities. They suggested that their study would have been stronger if predator densities had been assessed; we add that it would have been more powerful if the collembolan signal was also measured in predators in addition to that of aphids. Such multiple assays are now possible in the aphid–Collembola system described with the availability of collembolan PCR primers (Agustí et al. 2003a).

In the pear psylla system, such source sink relationships may be found if predators develop in nonorchard habitats or the orchard ground cover, and then colonize pear trees to feed on psylla. The abundance and diversity of predators associated with pear psylla in

orchards are known to be influenced by proximity to surrounding habitats (Miliczky and Horton 2005) and to how the orchard understory is managed (Fye 1983, Horton et al. 2003). These studies suggest that modification of pear orchards to incorporate elements of feral habitats, such as broad leaf plants that harbor alternate prey for predators, may lead to increased biological control of pear psylla. Recent studies testing the value of legume understory to enhance aphids in the ground cover suggest there is an increase of predator diversity and abundance in the understory that then spills over into the tree canopy (D.R.H. and T.R.U., unpublished data). Ongoing studies are using MAb34 developed here for predator GCA in pear orchards with and without enriched understory, together with a careful monitoring of predator and psylla densities in the pear trees. In support of these studies, we are developing antibodies to the supplemental aphid host in the legume-rich understory, the pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), and have developed PCR primers for pea aphid (T.R.U. and D.R.H., unpublished data). We are optimistic that the combined use of primary and alternate prey antibodies in GCA will allow us to identify prey preference profiles throughout the season and with accurate population density data gain a much clearer picture of the contribution of understory management to pear psylla biological control.

Acknowledgments

We have benefited from the help of many individuals through the several years of this work. Financial support was provided by the Winter Pear Control Committee and the Washington State Tree Fruit Research Commission. Expert guidance and technical support of the monoclonal creation and isolation and ascites production was provided by Mary-Jo Hamilton of the WSU Monoclonal Antibody Center (DMVP). Statistical advice and implementation of the PROC GLIMMIX analysis was provided by Bruce Mackey, Statistician General, USDA—ARS, Albany, CA. Valuable technical assistance was provided by Mike Clinton, Deb Broers, Merilee Bayer, Kelly Thomsen-Archer, and Pablo Palmandez. Earlier drafts of the manuscript benefited from reviews by Nina M. Barcenas, Heritage University, Toppenish, WA; Steven Arthurs, USDA–ARS Wapato, WA; and James Hagler, USDA–ARS, Maricopa, AZ. An anonymous reviewer provided insightful suggestions that improved the manuscript.

References Cited

Agustí, N., S. P. Shayler, J. D. Harwood, I. P. Vaughan, K. D. Sunderland, and W.O.C. Symondson. 2003a. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. Mol. Ecol. 12: 3467–3475.

Agustí, N., T. R. Unruh, and S. C. Welter. 2003b. Detecting *Cacopsylla pyricola* (Hemiptera: Psyllidae) in predator guts using CO1 mitochondrial markers. Bull. Entomol. Res. 93: 179–185.

Bradford, M. M. 1976. A dye binding assay for protein. Anal. Biochem. 72: 248–254.

Brunner, J. F., and E. C. Burts. 1975. Searching behavior and growth rates of *Anthocoris nemoralis* (Hemiptera: Anthocoridae), a predator of the pear psylla, *Psylla pyricola.* Ann. Entomol. Soc. Am. 68: 311–315.

Cohen, A. 1995. Extra-oral digestion in predacious terrestrial Arthropoda. Ann. Rev. Ent. 40: 85–105.

Dempster, J. P. 1960. A quantitative study of the predators on the eggs and larvae of the broom beetle, *Phytodecta olivacea* Forster, using the precipitin test. J. Anim. Ecol. 29: 149–167.

Faivre-D'Arcier, F., P. Millot, and L. P. Belzunces. 2001. Lachers inoculatifs *d'Anthocoris nemoralis* F en verger de poiriers. Phytoma 544: 76–78.

Fournier, V., J. R. Hagler, K. Daane, J. de Leon, R. Groves, H. Costa, and T. J. Henneberry. 2006. Development and application of glassy-winged and smoke-tree sharpshooter egg-specific predator gut content ELISA. Biol. Control 37: 108–118.

Fye, R. E. 1983. Cover crop manipulation for building pear psylla (Homoptera: Psyllidae) predator populations in pear orchards. J. Econ. Entomol. 76: 306–310.

Greenstone, M. H. 1996. Serological analysis of arthropod predation: past, present and future, pp. 265–300. *In* W.O.C. Symondson and J. E. Liddell [eds.], The ecology of agricultural pests. Biochemical approaches. Chapman & Hall, London, England.

Greenstone, M. H., and C. E. Morgan. 1989. Predation on *Heliothis zea* (Lepidoptera: Noctuidae): an instar-specific ELISA for stomach analysis. Ann. Entomol. Soc. Am. 82: 45–49.

Gut, L. J., C. Jochums, P. H. Westigard, and W. J. Liss. 1982. Variation in pear psylla, *Psylla pryicola* Foerster, densities in southern Oregon orchards and its implications. Acta Hortic. 124: 101–111.

Hagler, J. R., A. C. Cohen, D. Bradley-Dunlop, and E. J. Enriquez. 1992. Field evaluation of predation on *Lygus hesperus* using a species and stage-specific monoclonal antibody. Environ. Entomol. 21: 896–900.

Hagler, J. R., A. G. Brower, Z. Tu, D. N. Byrne, D. Bradley-Dunlop, and E. J. Enriquez. 1993. Development of a monoclonal antibody to detect predation of the sweetpotato whitefly, *Bemisia tabaci.* Entomol. Exp. Appl. 68: 231–236.

Hagler, J. R., and S. E. Naranjo. 1994. Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. Entomol. Exp. Appl. 72: 59–66.

Hagler, J. R., and S. E. Naranjo. 1996. Using gut content immunoassays to evaluate predaceous biological control agents: a case study, pp. 383–399. *In* W.O.C. Symondson and J. E. Liddell [eds.], The ecology of agricultural pests: biochemical approaches. Chapman & Hall, London, England.

Hagler, J. R., and S. E. Naranjo. 1997. Measuring the sensitivity of an indirect predator gut content ELISA: detectability of prey remains in relation to predator species, temperature, time, and meal size. Biol. Control 9: 112–119.

Hagler, J. R., and S. E. Naranjo. 2005. Use of a gut content ELISA to detect whitefly predator feeding activity after field exposure to different insecticide treatments. Biocontrol Sci. Technol. 15: 321–339.

Hagler, J. R., S. E. Naranjo, D. Bradley-Dunlop, F. J. Enriquez, and T. J. Henneberry. 1994. A monoclonal antibody to pink bollworm egg antigen: a tool for predator gut analysis. Ann. Entomol. Soc. Am. 87: 85–90.

Hagler, J. R., S. E. Naranjo, M. L. Erickson, S. A. Machtley, and S. F. Wright. 1997. Immunological examinations of species variability in predator gut content assays: effect of predator:prey protein ratio on immunoassay sensitivity. Biol. Control 9: 120–128.

Hansen, J. D., and P. A. Anderson. 2006. Mass rearing codling moths: improvements and modifications. J. Entomol. Soc. Br. Columbia 103: 33–36.

Hansen, R. R. 1975. Feeding rates and behavior of some predators of two *Psylla spp.* and a biology of a parasite of *Psylla pyricola* Förster. M.S. thesis, University of California, Berkeley.

Harwood, J. D., and J. J. Obrycki. 2005. Quantifying aphid predation rates of generalist predators in the field. Eur. J. Entomol. 102: 335–350.

Harwood, J. D., K. D. Sunderland, and W.O.C. Symondson. 2004. Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. Mol. Ecol. 13: 3549–3560.

Harwood, J. D., M. R. Bostrom, E. E. Hladilek, D. H. Wise, and J. J. Obrycki. 2007. An order-specific monoclonal antibody to Diptera reveals the impact of alternative prey on spider feeding behavior in a complex food web. Biol. Control 41: 397–407.

Harwood, J. D., S. W. Phillips, K. D. Sunderland, and W.O.C. Symondson. 2001. Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. Mol. Ecol. 10: 2049–2057.

Herard, F. 1986. Annotated list of the entomophagous complex associated with pear psylla, *Psylla pyricola* (Hom.: Psyllidae) in France. Agronomie 6: 1–34.

Horton, D. R., and T. M. Lewis. 2000. Seasonal distribution of *Anthocoris* spp. and *Deraeocoris brevis* (Heteroptera: Anthocoridae, Miridae) in orchard and non-orchard habitats of central Washington. Ann. Entomol. Soc. Am. 93: 476–485.

Horton, D. R., and T. R. Unruh. 2007. Pear insects: ecology and control, pp. 474–477. *In* D. Pimentel [ed.], Encyclopedia of pest management. Vol. II. CRC Press, New York.

Horton, D. R., D. A. Broers, T. Hinojosa, T. M. Lewis, E. R. Miliczky, and R. R. Lewis. 2002. Diversity and phenology of predatory arthropods overwintering in cardboard bands placed in pear and apple orchards of central Washington state. Ann. Entomol. Soc. Am. 95: 469–480.

Horton, D. R., D. Granatstein, R. S. Zack, T. R. Unruh, A. R. Moldenke, and J. J. Brown. 2003. Frequency of mowing and effect on density of natural enemies in the ground cover, in the tree canopy, and on the orchard floor at three Pacific Northwest pear orchards. Entomol. Exp. Appl. 106: 135–145.

Lenfant, C., A. Lyoussoufi, X. Chen, F. Faivre d'Arcier, and B. Sauphanor. 1994. Potential of *Forficula auricularia* L. as predator of pear psylla *Cacopsylla pyri* (L.) Entomol. Exp. Appl. 73: 51–60.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for mixed models. SAS Institute, Cary, NC.

Lovei, G. L., E. Monostori, and I. Ando. 1985. Digestion rate in relation to starvation in the larva of a carabid predator, *Poecilus cupreus.* Entomol. Exp. Appl. 37: 123–127.

Lovei, G. L., P. Sopp, and K. D. Sunderland. 1987. The effect of mixed feeding on the digestion of the carabid *Bembidion lampros.* Acta Phytopathol. Entomol. Hung. 22: 403–408.

Lovei, G. L., P. Sopp, and K. D. Sunderland. 1990. Digestion rate in relation to alternative feeding in three species of polyphagous predators. Ecol. Entomol. 15: 293–300.

Luck, R., B. Shepard, and P. Kenmore. 1988. Experimental methods for evaluating arthropod natural enemies. Annu. Rev. Entomol. 33: 367–392.

Madsen, H. F., P. H. Westigard, and R. L. Sisson. 1963. Observations on the natural control of the pear psylla, *Psylla pyricola* Förster, in California. Can. Entomol. 95: 837–844.

McMullen, R. D., and C. Jong. 1967. New records and discussion of predators of the pear psylla, *Psylla pyricola* Förster, in Br. Columbia. J. Entomol. Soc. Br. Columbia 64: 35–40.

Miliczky, E. R., and D. R. Horton. 2005. Densities of beneficial arthropods within pear and apple orchards affected by distance from adjacent native habitat and association of natural enemies with extra-orchard host plants. Biol. Control 33: 249–259.

Naranjo, S. E., and J. R. Hagler. 2001. Towards the quantification of predation with predator gut immunoassays: a new approach integrating functional response behavior. Biol. Control 20: 175–189.

Naranjo, S. E., and J. R. Hagler. 1998. Characterizing and estimating the impact of heteropteran predation, pp. 170–197. *In* M. Coll and J. Ruberson [eds.], Predatory Heteroptera: their ecology and use in biological control. Thomas Say Symposium Proceedings, Entomological Society of America, Lanham, MD.

Nickel, J. L., J. T. Shimizu, and T.T.Y. Wong. 1965. Studies on natural control of pear psylla in California. J. Econ. Entomol. 58: 970–976.

Pfannenstiel, R. S., and K. V. Yeargan. 2002. Identification and diel activity patterns of predators attacking *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in soybean and sweet corn. Environ. Entomol. 31: 232–241.

Riedl, H., P. W. Westigard, R. S. Bethell, and J. E. DeTar. 1981. Problems with chemical control of pear psylla. Calif. Agric. 35: 7–9.

Rieux, R., G. Fauvel, D. A. Faivre, G. Fournage, and W. A. Lyoussou. 1994. Study of biological control against *Cacopsylla pyri* (L.) in pear orchards by experimental release of *Anthocoris nemoralis* F. in the egg stage. II. Results and discussion. Bulletin OILB SROP 17: 120–124.

SAS Institute. 2002–2004. Help and documentation, version 9. 1. 3. SAS Institute, Cary, NC.

Sauphanor, B., L. Chabrol, F. Faivre d'Arcier, F. Sureau, and C. Lenfant. 1993. Side effects of diflubenzuron on a pear psylla predator: *Forficula auricularia.* Biocontrol 38: 163–174.

Sheppard, S. K., J. Bell, K. D. Sunderland, J. Fenlon, D. Skervin, and W.O.C. Symondson. 2005. Detection of secondary predation by PCR analyses of the gut contents of invertebrate generalist predators. Mol. Ecol. 14: 4461–4468.

Sheppard, S. K., and J. D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. Funct. Ecol. 19: 751–762.

Sigsgaard, L., P. Esbjerg, and H. Philipsen. 2006. Experimental releases of *Anthocoris nemoralis* F. and *Anthocoris nemorum* (L.) (Heteroptera: Anthocoridae) against the pear psyllids *Cacopsylla pyri* L. (Homoptera: Psyllidae) in pear. Biol. Control 39: 87–95.

Solomon, M. G., J. E. Cranham, M. A. Easterbrook, and J. D. Fitzgerald. 1989. Control of the pear psyllid, *Cacopsylla pyricola* in South East Engl. by predators and pesticides. Crop Prot. 8: 197–205.

Sopp, P. I., K. D. Sunderland, J. S. Fenlon, and S. D. Wratten. 1992. An improved quantitative method for estimating invertebrate predation in the field using an enzyme-linked immunosorbent assay. J. Appl. Ecol. 29: 295–302.

Sutula, C. L., J. M. Gillett, S. M. Morrissey, and D. C. Ramsdell. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70: 722–726.

Symondson, W. 2002. Molecular identification of prey in predator diets. Mol. Ecol. 11: 627–641.

Unruh, T., and B. Higbee. 1994. Release of laboratory reared predators of pear psylla demonstrates their importance in pest suppression. IOBC/WPRS Bull. 17: 146–150.

Unruh, T., P. Westigard, and K. Hagen. 1994. Pear psylla, pp. 95–100. *In* J. Nechols [ed.], Biological control in the western United States. Department of Natural Resources Publ. 3361, University of California, Oakland, CA.

Westigard, P. H., L. G. Gentner, and D. W. Berry. 1968. Present status of biological control of the pear psylla in southern Oregon. J. Econ. Entomol. 61: 740–743.

Received 1 October 2007; accepted 28 April 2008.